The preparation and treatment of woods for microscopic study*

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(WITH SIX TEXT FIGURES)

CONTENTS

1. INTRODUCTION

By reason of extreme variation in the mineral, resin- or gum-like content, and in the texture, hardness, and other properties peculiar to the stem tissues of tropical woody plants, the task of preparing these for microscopic study is ordinarily a difficult one, particularly in the case of an extensive series composed of numerous unrelated species of widely differing ecological types. The published technique relating to the preparation of wood for slides has been worked out primarily from a study of the comparatively soft

* Contribution from the Osborn Botanical Laboratory.

tissues of the woods of temperate regions, to which anatomical work has, in large part, been confined. Such tissues often cut satisfactorily without treatment, while even oak and hickory, among the hardest of such woods, if taken from fresh (green) material, may be satisfactorily sectioned along any plane, by a microtome, without any preparatory treatment. Tropical wood collections, on the other hand, ordinarily embrace a high proportion of species the tissues of which, particularly when selected from dry material, can only be cut after a more or less prolonged treatment, the nature of which varies considerably according to the structural features of the species or type. Moreover, after sections of sufficient clearness have been obtained, to an even greater degree than in temperate woods, there are many anatomical characters, often of the greatest interest, which cannot be satisfactorily observed without the use of reagents, stains, or media of definite refractive properties. To meet the particular needs in this field, there is insufficient information at hand, and, further, little seems to have been added in recent years. Papers marking distinct progress in anatomical work too often neglect to publish essential details in the methods employed.

During the years 1916–1918, the writer carried out an extended investigation of the woods of Hawaii. In the course of these studies it was found necessary to devote a very considerable amount of the time to the preparation of slides, and altogether more than three thousand permanent mounts were made, including approximately one hundred macerations. In connection with this work, various accepted methods were tested and a number of new ones were devised. In the present paper, the technique employed is described in some detail, particular attention being called to certain heretofore undescribed methods of treatment and to a number of improvements on the methods in in common use. While emphasis is thus placed upon the treatment of the highly complex woody tissues of tropical dicotyledons, the suggestions embodied should be of value in the treatment of other woods than tropical, or of other than woody tissues, in that, to a certain extent, the treatment of some of the cellulose tissues of the stem, particularly the collenchyma and phloem layers, has been included.

The writer is indebted to Professors J. W. Toumey, S. J. Record and others, of the Yale School of Forestry, and to Professors A. W. Evans and G. E. Nichols, of the Department of Botany, for the use of authentic material, for the facilities essential for accurate work, and for many helpful suggestions and criticisms.

2. ADJUSTMENT OF MICROTOME

For sectioning, Thomson's modification of the Jung-Thoma sliding microtome ('10), supplied with Walb blades 170 mm. in length and 35 mm. in width, was used. To secure the best results, it was found essential that careful attention be paid both to the sharpening of the knife and to its adjustment on the carriage of the microtome. Briefly, the knife was first sharpened to a wedge-shaped edge of which the two planes were inclined at an angle θ (FIG. 1) of 20° to each other. At the same time, perfect axial

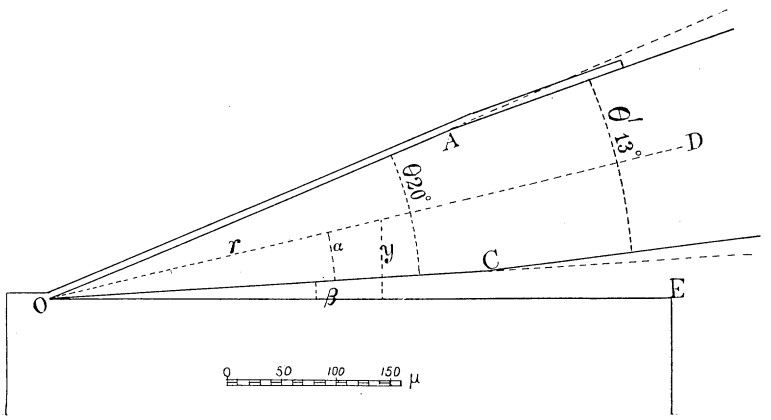

FIG. 1. Diagram to show edge of sliding microtome knife cutting section 10 μ thick. β (representing downward inclination) = 4.° θ (representing acuteness of edge after being ground with back raised by cylindrical clamp) = 20.°

alignment of the cutting edge was secured in the manner to be described presently. In mounting on the carriage, the blade was first inclined downward so as to form an angle β (FIG. 1) of 4° between the lower plane (*OC*) of the knife wedge and *OE*, the plane in which a given tissue is to be sectioned (vertical inclination). The knife carriage was then revolved in a horizontal

plane so that the edge of the blade lay at a definite angle with reference to the direction of movement of the carriage on the slideway. For general work, a horizontal angle of inclination of about 20° was found satisfactory, but to obtain extremely thin sections, as in imbedded material, an angle of about 5° proved to be best.

Since angle θ' (FIG. 1), which the planes of the average microtome knife make with one another, is ordinarily more acute than at the edge honed to 20°, this difference must be allowed for in adjusting the vernier. Thus if θ' is 13°, the vernier should read 7.5° to give an inclination of 4°, an excellent angle for most work. A few woods containing gummy or resin-like material cut better at a somewhat greater inclination, as 6°. On the other hand, if the blade tends to "pull into" the tissue, a more acute inclination may be needed.

It is doubtful if cutting edges with θ less than 20° should ever be used in cutting woody tissues. The more acute edge is too easily injured to be used in cutting many of the harder woods, such as *Pandanus*, even when these have been carefully treated. The 20° edge, on the other hand, has sufficient strength in blades of good quality to appear undamaged under the microscope after use in cutting any of the treated material, and at the same time it is sufficiently acute to meet every requirement. The chief objection to using a more obtuse edge is that the section, especially at steep inclinations of the blade, is too sharply bent at *O*, FIG. 1, in cutting, so that it tends to curl or even roll up. It is partly for this reason, also, that the downward inclination of the knife (angle β) should not be greater than necessary.

But even if the inclination is correct, the quality of the work which the instrument is capable of doing may be seriously impaired by an imperfectly aligned cutting edge. This, of course, is a point which does not have to be taken into account at all in using a rotary microtome. For the sliding microtome, it is not sufficient that the edge shall coincide with a single plane, as *OD*, FIG. 1. Errors of alignment from heel to point may still be present by reason of which an otherwise well sharpened edge would not coincide with a plane passing through *O* at right angles to *OD*. Here an error so slight as to escape detection by the eye may be the entire cause for poor work. For example, let the error of

alignment, r, midway between heel and point be 0.3 mm. with reference to a plane meeting the bisecting plane OD at right angles and in contact with heel and point. Let angle $\theta = 20°$, angle $\alpha = 10°$, angle $\beta = 4°$, and angle $\alpha + \beta = 14°$. Distance $y = r \sin (\alpha + \beta)$, representing the error of alignment of the knife edge with reference to the plane of section, will then be 72 μ. In other words, when heel and point of blade are in contact with the plane of section, the edge at mid-distance will be 72 μ above this plane; whence it follows that with each full heel-to-point horizontal stroke in cutting, as when revolved horizontally at an acute angle of about 4° with reference to the direction of motion, the edge will twice traverse a vertical distance of 72 μ through the tissue, destroying the section. With the edge thus imperfectly aligned, the knife can be used to advantage in a sliding microtome only when placed less obliquely to the direction of motion than is essential for the cutting of thin sections, as at 20°–40°, in which position sections are cut with fractional strokes considerably less than the length of a 170 mm. blade.

To give the true edge essential to accurate work in cutting woody tissues, the use of a special type of hone is necessary. Such a hone consists of a piece of plate glass as wide as the knife is long, with a true plane surface covered with Diamantine Powder and oil or water, so that the knife remains constantly in contact with the hone throughout its length during the process of grinding. An excellent abrasive may be prepared by grinding two fine Belgian or carborundum hones together, and collecting the fine powder thus produced on the surface of the glass. This is not so harsh as the No. 1 Diamantine. Having ground the blade on the glass hone until the edge at both sides makes perfect contact with the surface, a brief grinding on a Belgian hone (2 x 8 in.) wet with 30 per cent glycerine will often give an edge sufficiently even and sharp for general work. The knife should be drawn across the hone obliquely, heel and edge forward, alternately upon each side. But, for best results, this grinding should be followed by sharpening upon a flat leather-surface hone, the blade in this case being pushed obliquely, point and back forward, along the hone. The surface of this hone may be kept in condition by the use of any of the fine abrasives used for this purpose on razor strops. A strop

of the hanging type should not be used in that it tends to round the planes of the knife wedge. The wedge angle is best regulated by the use of a cylindrical metal clamp fastened to the back of the knife during the process of sharpening.

In addition to the possession of (1) true axial alignment, the edge should (2) be sharp enough throughout its length to cut a hair by contact and (3) should appear perfect'y even and without nicks under the low power of the microscope. The number of sections which may be cut without re-sharpening the knife is ordinarily small; with difficult materia!, frequently as few as two or three perfect sections.

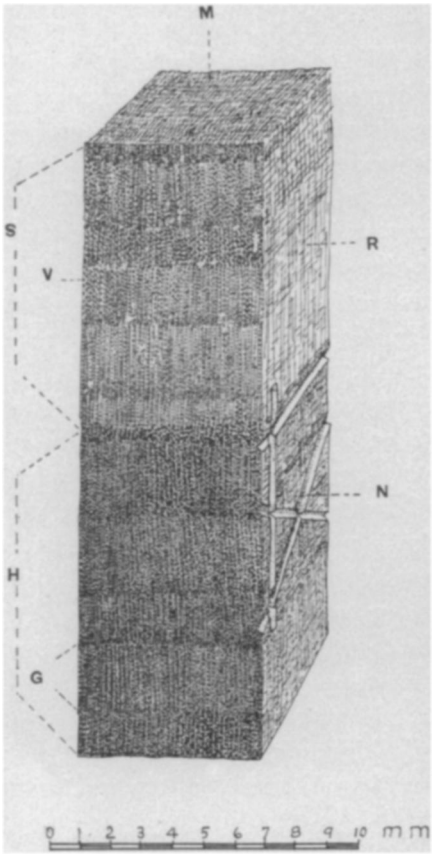

FIG. 2. Block showing tropical wood (*Tecoma* sp.) with tissues properly oriented for sectioning in cross, radial, or tangential planes. *H*, heart-wood; *S*, sap-wood. The rays (*M*, *R*) are low and narrow; the vessels (*V*), numerous and small. *G*, growth-ring.

3. PREPARATION OF MATERIAL FOR SECTIONING

A. CUTTING THE BLOCKS

In preparing material for sectioning, the blocks must be shaped with reference to the plane or planes in which the sections are to be cut. Thus a block intended for a cross, radial, and tangential series of sections may have a radial length of 2 cm., a vertical height of 8 mm., and a tangential thickness of 6 mm. Sections cut from the three planes of such a block will ordinarily include at least one full growth-ring (*G*, FIG. 2) and the full height of the

rays. Occasionally a species with higher rays or other special features may require blocks of larger dimensions. When present, both heart-wood (*H*) and sap-wood (*S*) may be included. The block should be carefully trimmed with a knife so that the tissues are perfectly oriented with respect to each of the planes intended for sectioning. The medullary rays in particular should coincide as perfectly as possible with the radial plane. In tropical woods, the rays are often narrow, visible only under a lens, and curved, so that more than ordinary care is necessary in trimming this surface.

Before proceeding with the treatment, the blocks should be numbered for sake of record. Some use a system of notches along the edges. Perhaps one of the best methods is to carve Roman numerals on the radial face not intended for sectioning, underscoring IX to distinguish it from XI (*N*, FIG. 2); also, many tropical woods require an arrow to indicate the direction of growth.

B. REMOVING THE AIR

After the blocks have been cut the desired shape, air should be extracted as far as possible from the cell lumina by alternate boiling and cooling in water. The use of an Eimer and Amend aspirator No. 3250 after boiling greatly hastens the process. This treatment should be continued until the lightest blocks sink and little or no air comes from the tissues when the aspirator is applied. Most blocks sink in a few hours, but species with numerous tyloses may take a longer time. Thus, blocks of *Rhus semialata* Murr. var. *sandwicensis* Gray remain floating five days during the boiling and cooling process, or two days when boiling periods are followed by the application of the aspirator.

C. SOFTENING THE TISSUES

Nearly all xerophytic species of tropical woods require a long treatment in strong hydrofluoric acid, often extending over several weeks, before they can be sectioned. Rain forest and bottom-land woods are frequently soft, but even such woods usually cut with clearer outlines when treated for at least a few days in acid.

For treating material with hydrofluoric acid, the blocks, after removal of air, may be placed in a wide-mouthed glass bottle, care-

fully coated inside with hard paraffin, or, still better, in cups prepared by cutting empty hydrofluoric acid bottles. Strong hydrofluoric acid is added to cover the material, and the container then corked or covered with a hard paraffin plate for a period varying from a few days to six weeks or longer, the length of time being determined by removing blocks at intervals of one to several days and testing with a sharp scalpel until they are found to be sufficiently soft to cut easily in transverse section.

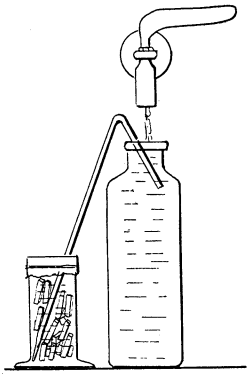

FIG. 3. Siphon apparatus for washing blocks or sections.

In the writer's experience, tissues are rarely injured by leaving them a long time in hydrofluoric acid. Thus, in case of a young stem of *Dracaena curea* Mann, delicate tissues, such as the phloem, cambium, undifferentiated parenchyma, and other thin-walled tissues, together with the bundles of needle-like raphides of calcium oxalate, were all left uninjured by long treatment in acid. In one instance, a block of *Santalum Freycinetianum* Gaud., of the size and shape described for FIG. 2, was placed in acid on September 6, 1917, and left until March 26, 1918, when it was washed and sectioned. Though in moderately strong acid for over six months, all structures, including the mineral crystals, were in perfect condition.

Hydrofluoric acid probably softens the tissues mainly, if not entirely, by the removal of silica (desilicification). But other minerals would probably be acted upon. For example, calcium would form the insoluble calcium fluoride which would remain in the wall. Curiously, crystals of calcium oxalate in crystal parenchyma or idioblasts usually remain nearly or quite intact long after the wood has been sufficiently softened to cut well. Even in maceration by Schultze's solution, crystals may be uninjured. Dr. A. J. Hill suggests that, in case of the hydrofluoric acid treatment, the crystals may have been protected by the formation of an insoluble film of calcium fluoride, the possibility of which is seen from the following equation:

$$CaC_2O_4 + 2\,HF = \underset{\text{Insol.}}{CaF_2} + H_2C_2O_4$$

The presence of such a film was indicated, though not certainly proven, by refraction tests. Another possible agency of protection may consist in a thin and presumably impermeable organic membrane closely applied to the surface of the crystal. Such a membrane may be detected by zinc chlor-iodide or by the haematoxylin stain. Also, a thicker mucilaginous membrane is usually visible outside the inner thin membrane. The resistant character of these organic coverings is indicated from the fact they may be little if at all acted upon by Schultze's solution.

As soon as the tissues have been sufficiently softened to cut well, they should be washed in running water for about four days to remove all acid. An arrangement like that shown in FIG. 3 is recommended both for this purpose and for washing the sections at a later stage. The blocks are placed in a short jar the top of which is covered with cheese-cloth through which the pointed end of a glass siphon tube is pushed. The long arm of the siphon should extend to the bottom of the jar. The short arm takes its water from a somewhat taller jar placed beneath a running tap. After washing, the blocks should be covered with glycerine where they may remain until needed for sectioning. The effect of the glycerine is such that any tissues which have become too brittle, in a few hours, become sufficiently flexible to cut well with the microtome.

D. IMBEDDING

Celloidin method.—Certain woods of peculiar structure, like *Pisonia*, in which portions are composed of very soft unlignified tissues, and other woods, in case it is desired to cut extremely thin sections, must be imbedded in celloidin before cutting. In imbedding, the general procedure followed was that described by Plowman ('04). Solutions of Schering's celloidin in a mixture composed of equal volumes of ether and absolute alcohol may be prepared in 2 x 8 cm. shell vials in concentrations of 2, 4, 6, 8, 10, 12, 14, 16, 18, and 20 per cent. The blocks to be imbedded are first treated in hydrofluoric acid and washed as above described. Next, in the usual manner, they are gradually transferred to 100 per cent alcohol. During the latter stages of this dehydration process, they are left two days in each alcohol, and the absolute alcohol is changed at least once. They are now transferred to a

mixture of ether and alcohol, in equal volumes, and finally through the graded series of celloidin solutions, being left about twenty-four hours in each concentration. On reaching the 20 per cent solution, a pressure-resisting container should be used. A brass case with screw top, such as a microscope objective box, will answer the purpose, or the corked imbedding vial may be wrapped tightly with strong cord. After adding sufficient solid celloidin to thicken the solution as much as possible, it is placed in a paraffin oven at 50–60° C. for three days. During this time, the contents of the vial are kept under pressure by the confined gases with the object of forcing the celloidin more perfectly into the tissues. The imbedded blocks are then removed, hardened in chloroform for twelve to twenty-four hours, and placed in a mixture of equal parts of 95 per cent alcohol and glycerine until ready for sectioning.

Paraffin method.—Only very soft tissues should be imbedded in paraffin. Woody tissues, even after being well softened by acid, are likely to become too hard during the process of imbedding in paraffin to cut well with the microtome.

E. SECTIONING WITH THE MICROTOME

Unimbedded material.—After softening the tissues as already described, the material is ready to section. In cutting sections, the blade of the knife should be kept flooded with a 15 per cent solution of glycerine in 95 per cent alcohol. Sections are best removed from the blade by a fine camel's hair brush and are transferred to water. From blocks prepared as above described, cross-sections may be cut as thin as 10–15 μ; radial sections, 8–20 μ; and tangential sections about 7 μ. Where, for special purposes, such as the study of the detailed structure of pit membranes, it is necessary that the sections be considerably thinner than this, the material must be imbedded in celloidin as above described.

Imbedded material.—To section material after imbedding in celloidin, the blade may be moistened either with 95 per cent alcohol or with the alcohol-glycerine mixture. Sections of woody tissues may then be cut to less than 3 μ in thickness. To obtain very thin sections, it is essential not only that the knife be sharp, but that it be ground to an alignment sufficiently true to admit of the use of the blade at very oblique horizontal inclinations with refer-

ence to the direction of motion, such as 4°. Thin sections such as these require great care in handling. In most cases, particularly in the longitudinal planes, only extremely small pieces may be cut, since such sections, being less than the diameter of the cells, are often sections of single cells and may even be too small to be seen without a lens.

After washing the sections in water to remove all glycerine, the celloidin may be removed by covering the sections for several hours with the ether-alcohol mixture. The process of clearing may go on very slowly and more than twenty-four hours may be required to remove all celloidin. The sections should now be placed on a slide in dilute albumen fixative (one drop to 2 c.c. of distilled water) and warmed slightly until dry. After standing over night, they are ready for staining. For staining, Koplin jars are best used, but otherwise the procedure is the same as for unimbedded material to be described in connection with differential stains.

F. FREE HAND SECTIONS

Since small unstained radial and tangential sections mounted in glycerine ordinarily show the essential anatomical features, the following method, though especially adapted to coniferous woods, is suggested, in connection with macerated preparations, to assist in the rapid identification of material. The radial and tangential surfaces are first cut true with a knife, then a sharp razor is drawn lightly over the surface allowing it to cut thin fragmentary pieces. These are placed in a drop of water on a slide and held over a flame until boiling temperature is reached. Alcohol is now dropped upon the section until all air has disappeared from the cell-lumina. After a brief staining in aqueous potassium iodide solution of iodine, the sections are ready to mount in glycerine.

4. PREPARATION OF MATERIAL BY MACERATION

Many tropical woods contain substances in the ray cells and in other elements, by reason of which details of pitting and similar features are obscured. Macerations will be found satisfactory in such instances and the following modification of Schultze's method is suggested for their preparation:—

1. Cut chips to expose a radial length of 2 cm., an axial length of 1 cm., and a tangential thickness of 2 mm.

2. Place these in a numbered test tube, cover with water, and keep at boiling temperature in the water bath for about an hour. The hot water is then replaced by fresh cold water and the tube reheated. Alternate cooling and boiling periods should be continued for at least five changes.

3. Cover the material with 50 per cent nitric acid and add a small amount of potassium chlorate (as much as may be taken upon the point of a small knife blade). It is now kept at boiling temperature until the pieces have whitened and commenced to fray, when cold water is poured on, causing the macerated material to settle. The acid solution is then replaced with water and allowed to heat with the purpose of removing the acid solution.

4. Transfer the material to a watch-glass where it may be teased apart with a small size artist's brush. By means of a pipette, change the water several times or until all particles of foreign matter and traces of chemicals have been removed.

5. Replace the water by 95 per cent alcohol for 30 minutes or until all air has been removed from the cell-lumina.

6. Replace the alcohol by a 2 per cent solution of Bismarck brown in 70 per cent alcohol, in which the material should stand for twelve to twenty-four hours.

7. To remove excess stain, wash quickly in alcohol and transfer to glycerine, which should be changed if greatly colored by the stain.

8. Mount in glycerine and cement with Brunswick black or gold size.

Preparations such as these are almost essential for the working out of the details of vascular anatomy. If a water bath capable of receiving twenty test tubes is used, but little time need be consumed in making the preparations. The action of the chemicals proceeds so slowly that there is little danger of tissues being destroyed.

5. DEFINITION OF ANATOMICAL DETAILS

A. BY DIFFERENTIAL STAINING

In the writer's experience, Haidenhain's iron-haematoxylin has proved the best all-round stain for bringing out the anatomical

structure of woody tissues. This is true both for the relatively thick sections cut from unimbedded blocks and for the very thin sections obtained by the celloidin method. In these latter sections, for example, it is possible with this stain to bring out such structures as the reticulated thickenings of pit membranes as well as similar features not visible by other means.

Before staining, sections cut from the unimbedded blocks or imbedded sections on slides are washed in three or more changes of water to remove glycerine. The sections are then covered with a 2.5 per cent aqueous solution of iron-alum (ferric ammonium sulphate) for five to twelve hours. Next, they are washed in running water for five minutes (or two minutes in case of slide material), stained with 0.5 per cent aqueous solution of haematoxylin (Chamberlain, '15, p. 41) for twelve to twenty-four hours, washed in water for three minutes, and then again treated with the iron-alum solution. The sections are left in the iron-alum until they become light gray, but, for best results, the material should be watched under the microscope, so that the process may be stopped the moment the clearest definition is reached. Sections are then washed in running water for three hours or longer to remove all trace of iron-alum. In many cases, this washing also serves to remove precipitates and other clouding matter from the tissues so that the clearness and transparency of the section is greatly improved. It is often well to extend the period of washing to twenty-four hours or longer. For this purpose the siphon apparatus shown in FIG. 3 is useful, since it allows a thorough percolation of water through the sections for any length of time without danger of mechanical injury to the tissues or of loss of material. The sections rise flat to the top directly in the outflowing tap water which, from a public supply system, is usually sufficiently alkaline to give a clear blue color to the haematoxylin, hence being preferable to distilled water for this purpose.

For best results, woody tissues stained as above should be counterstained with safranin. This stain is made up by combining equal portions of a 1 per cent solution of alcohol-soluble safranin in 95 per cent alcohol and a 1 per cent aqueous solution of water-soluble safranin. For extremely thin membranes, where a very weak counterstain is desirable, one to three minutes in the safranin

may be sufficient; but for general work, longer periods up to two hours, giving a heavy but not too opaque stain, are desirable. Dehydration is accomplished by washing quickly in 95 per cent alcohol, then successively in absolute alcohol and xylol. The sections are mounted in dammar or balsam.

Sections which tend to curl badly after cutting, as in case of many woods with thick-walled elements, may be placed, as soon as cut, between two glass slides and allowed to dry before staining. In most cases, the section may then be placed free in the stain, but occasionally it may be necessary to proceed with one or more steps of staining before removing from the slides.

For a number of purposes, the above process, with slight modification, may be used successfully in staining the soft cellulose tissues of the bark; but for this purpose Congo red is superior to safranin as a counterstain. Where shrinkage due to dehydration is not too great and the cell contents stain black, as in *Malus* or *Pyrus*, preparations showing clearly such details as the reticulate thickenings, pits, and even protoplasmic bridges through the pit-membranes of the hypodermal collenchyma may be obtained by the following process:—

1. Sections 6 μ in thickness are cut from green material collected in winter, to show pits and protoplasmic bridges in section, or 10 μ in thickness to show pits and reticulations in surface view.

2. Place for five hours in 2.5 per cent aqueous solution of iron-alum.

3. Wash five minutes.

4. Stain about twelve hours in 0.5 per cent aqueous solution of haematoxylin.

5. Wash five minutes.

6. Differentiate in a 2.5 per cent iron alum solution until sections are gray.

7. Wash three hours.

8. Stain in a saturated aqueous solution of Congo red one to five minutes for pits in section, ten to thirty minutes for pits in surface view, one to two hours for reticulation of thin end-walls.

9. Dehydrate in absolute alcohol one minute.

10. Clear in xylol one minute.

11. Mount in dammar.

Also, longitudinal sections of phloem 6 μ in thickness, when treated as above, will show the lattice-like arrangement of sieve areas in the lateral walls of the sieve tubes.

A convenient method of numbering and labeling slides is as follows. By means of a camel's hair brush, cover the entire upper surface of the slide outside the coverslip with dilute dammar (mounting consistency diluted twenty to thirty times with xylol). The brush is best fixed in the cork of a bottle used to contain the solution. In a few seconds the records may be written with Higgin's waterproof ink, and index arrows sketched to point to any special part of the mount, if desired. The surface should then again be brushed with dammar that the slide may be freely handled or washed without injury to the writing.

B. BY DIFFERENTIAL REFRACTION

The laws of refraction may be employed not only to give clear definition to details in outline, but also to determine physical or chemical qualities of cell-walls or cell-contents. In either case characters may often be determined by refraction more readily and with greater precision than by the reaction of stains or of chemical reagents. Refraction opens a wide field for research in plant histology and will be treated in some detail in a subsequent paper dealing with refraction of light in relation to plant tissues.

6. MICROCHEMICAL REACTIONS

Characters readily observed by microchemical means often have a systematic value, or are of ecological or physiological interest, or are closely identified with the peculiar qualities of color, hardness, hygroscopicity, porosity, durability, strength and other properties upon which depends the special value of any given wood in the arts. In the following paragraphs are indicated several important characters frequently found in woody tissues of warm climate trees, together with a number of microchemical tests helpful in connection with their demonstration.

A. CELLULOSE

Sections are placed twelve hours or longer in a solution made by adding one drop of concentrated aqueous Congo red to 20 c.c.

of distilled water. Cellulose tissues turn deep blue as soon as transferred to 10 per cent hydrochloric acid. If mounted in glycerine (acidulated by adding one drop of strong hydrochloric acid to 10 c.c. of glycerine) and well cemented with Brunswick black or gold size, the blue color will remain several months without fading.

B. CELLULOSE-LIGNIN

The above test for cellulose may be varied by transferring the section for a brief period to a strong aqueous solution of anilin chloride immediately after the treatment with Congo red, the remainder of the treatment being the same. Lignified tissues appear yellow; cellulose, blue.

C. GUMS

Gummy substances, both those which are soluble in water and those which swell without going into solution (mucilages), are constantly met with in woody tissues, occurring in the lumina of vessels, tracheids, and other cells, or in intercellular canals or cavities, these latter resembling resin cavities. Certain forms of each (gums and mucilages) are believed to play the rôle of reserve material (Haas and Hill, '13, p. 125; Grüss, '96). Fibers, composed in large part of mucilaginous inner layers, are also of frequent occurrence. The gummy nature of amorphous bodies observed in permanent mounts is usually indicated by shrinkage checks or other evidences of contraction on dehydration, as in FIG. 5 (see further below). Gums may be further distinguished from resins by their insolubility in chloroform.

Mucilaginous laminae in fibers ("gallertartige Verdickung" Sanio, '63, p. 105; Solereder, '08, p. 1143: "Hemicellulose," Grüss, '96; Schellenberg, '05: "cellulose," Potter, '04: "mucilaginous fibers," Sachs, '75, pp. 35–36; Jeffrey, '17).—Frequently woody fibers, particularly of xerophytic species, are composed of one or more clear, gelatin-like concentric inner layers which shrink greatly on drying (FIG. 4); in young tissue, they turn deep violet with zinc chlor-iodide. Solereder ('08, p. 1143) records twenty-two families in which such fibers occur, and his list is not complete. In a given wood, fibers of this description may occur either sporadically or in regular distribution;

they may be few in number or may compose nearly or quite 100 per cent of the prosenchyma. In some cases (*Robinia*), the purple reacting lamellae of fibers may function as reserve material, and be more or less completely dissolved in the growing season (Schellenberg, '05; Grüss, '96). In this connection, twigs of *Acer rubrum* L., *Cercis canadensis* L., and *Robinia Pseudo-Acacia* L., gathered in winter condition, sectioned, and treated with zinc chlor-iodide, make favorable material for study. A number of distinctive properties are exhibited by such fibers:

a. Hygroscopicity.—Mucilaginous layers readily take up water, swell without going into solution, and shrink correspondingly on

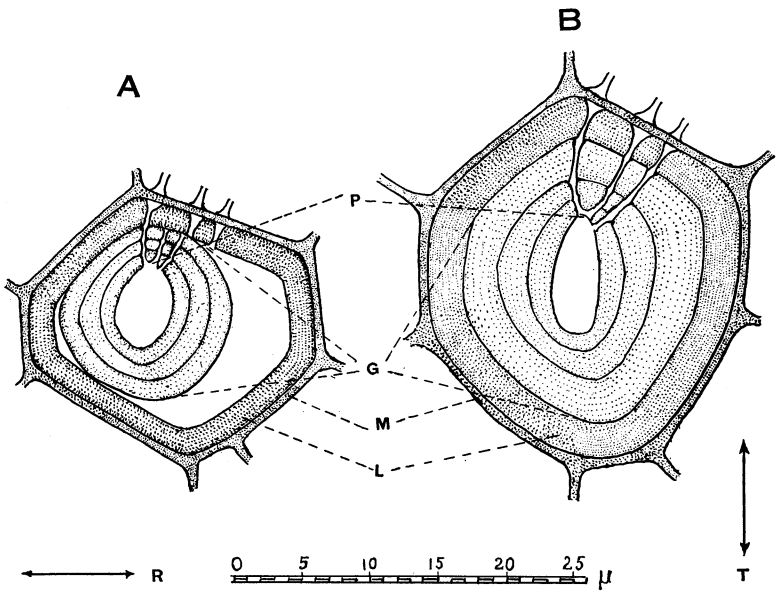

FIG. 4. Abbé camera drawings of a laminated fiber of *Xylosma hawaiiense* Seem. in cross-section. *A*, after desiccation. *B*, after treatment with water. All layers have swollen, but the mucilaginous layers (*G*) more than the ligno-cellulose outer portion (*L*). The simple pits (*P*) come into alignment after soaking. One of the pits remains permanently in connection. *T*, tangential direction. *R*, radial direction.

drying. If cross-sections 15–30 μ in thickness are mounted on a slide in water, then dehydrated by flowing absolute alcohol beneath the coverslip, the mucilaginous layers may be observed to shrink greatly, in the majority of cases, drawing away from the

thin outer ligno-cellulose layer usually present (FIG. 4, *A*). Fibers in which the mucilaginous layers remain in contact with the outer layers on drying ordinarily show large shrinkage checks extending radially outward from the center, through the mucilaginous portion. Dehydration may be completed by placing the slide upon the water bath for a few seconds. In stained sections mounted in balsam or dammar, mucilaginous membranes are usually shown in the contracted state. In glycerine, water, or other media in which the tissue has been mounted without dehydration, the mucilaginous layers appear in the swollen condition. Abbé camera outline sketches of single fibers before and after dehydration, made with high power and extended draw tube to obtain the greatest possible enlargement, accurately show the amount of shrinkage as in FIG. 4, *A* and *B*. The mucilaginous core of such fibers often contracts over 25 per cent of both radial and tangential dimensions on drying, and swells rapidly to original size on admission of water. The outer ligno-cellulose layer and the middle lamella (*M*) which is here indistinguishable from the primary thickening of the fiber, on the other hand, show relatively little change in dimensions. The shrinking and swelling of the mucilaginous thickenings is partly independent of the other parts of the tissue, so that, as in the case of the Hawaiian woods examined by the writer, the wood tissue itself was not observed to swell or shrink in proportion to that of the mucilaginous layers of the fibers. However, blocks of wood in which mucilaginous fibers were abundant, as, for example, *Xylosma*, were found to shrink as much as 12 per cent tangentially and 7 per cent radially on drying from saturation.

b. Reaction to stain and other reagents.—Stains and chemical reagents react differently according to the age of the tissue; also, after material has been softened in hydrofluoric acid, neither stains nor chemical reagents give characteristic reactions.

In old tissue (heartwood), haematoxylin, Bismarck brown, and anilin blue stain the mucilaginous layers with varying intensity as a substance of variable composition. Very commonly stains, particularly the safranin, are readily extracted by the alcohol washes or other treatments in the staining process, so that the mucilaginous layers appear slightly or not at all stained in the

finished mount. With phloroglucinol and hydrochloric acid, a more or less pronounced red reaction is usually obtained; with zinc chlor-ioidide, a yellow, brownish, or sometimes purplish color.

In young tissue (twigs, sapwood), the mucilaginous membranes stain deeply with haematoxylin, or Congo red; with zinc chlor-iodide they turn deep purple or violet.

c. Fracture.—In maceration, mucilaginous fibers are usually extremely brittle. A fragment ordinarily shows conchoidal fracture across the mucilaginous core. Woods in which mucilaginous fibers are abundant are likely to be brittle.

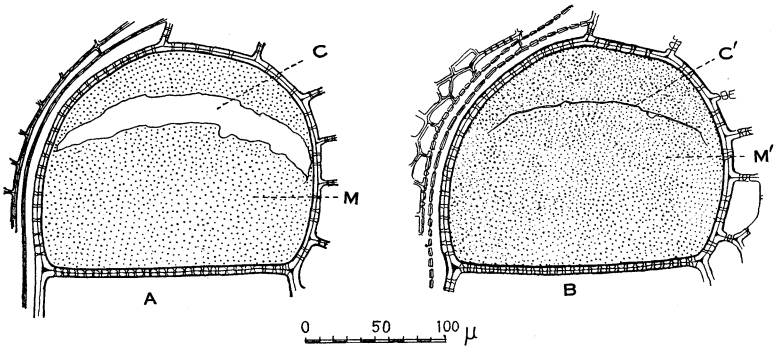

FIG. 5. Abbé camera drawings of vessel in *Tabebuia chrysantha* Nichols, (?), showing surface of insoluble gum (mucilaginous) plate (*M*). *A*, after dehydration; *B*, after admission of water beneath coverslip. The shrinkage crack (*C*) closes in *B*.

Mucilage or insoluble gum in cells, vessels, or canals.—The mucilaginous or gummy substances, which occasionally fill certain cells, vessels or intercellular cavities in dicotyledonous woods, respond to water tests in much the same way as the mucilaginous portion of mucilaginous fibers. Here the substance does not ordinarily pull away from the sides of the cavity or canal on dehydration, but cleaves apart in long wide gaps extending, usually, across the center. Such shrinkage cracks may be observed to close up tightly soon after admitting water at the edge of the coverslip, and drawing it through by means of blotting paper at the opposite side (FIG. 5). The water test serves also to distinguish the gums or mucilages from the resins, which, though they may shrink on dehydration, do not swell in water.

D. ESSENTIAL OILS, RESINS, AND GUM-RESINS

Substances of this description occur abundantly in woody tissues of tropical dicotyledons, in the cell lumina or in intercellular canals (Guignard, '92; Record, '18), in the form of globules, transverse plates or irregular masses. As before indicated, such substances may readily be distinguished from the gums by the fact that after drying they do not swell in water; also, by their solubility in chloroform. The gum-resins, however, may have some of the properties of each component. To determine the solubility in chloroform, sections (particularly those from seasoned blocks) should remain twenty-four hours in the solvent.

The essential oils, which often possess an aromatic odor, resemble the resins except that such bodies readily dissolve when absolute alcohol is drawn beneath the cover glass.

E. FATS

The fatty oils resemble, in appearance, the essential oils with which they often occur in the same cell. Both are stained by Sudan III. The fatty oi s are distinguished from essential oils from the fact that absolute alcohol dissolves few fatty oils, but does dissolve the essential oils; strong potassic hydrate saponifies fatty oils, but not essential oils, although some complex resins which break down into a fatty component may appear to be saponified; a temperature of 130° C. volatilizes essential oils but not the fats.

F. TANNIN

The presence of tannin in tissues is readily demonstrated by familiar tests. A bluish or greenish black is caused by a neutralized solution of ferric chloride, and a reddish yellow by ammonium molybdate.

G. MINERAL CRYSTALS

Vertical strands of short cells, each with a single crystal of calcium oxalate nearly filling the cavity, are of frequent occurrence in tropical woods, being found in over 26 per cent of the Hawaiian woods studied by writer. Less frequently they occur in ray-cells and other tissues. Their composition is determined from the fact that calcium oxalate dissolves without effervescence in

hydrochloric acid, but is unaffected by acetic acid. Calcium carbonate, which is of comparatively rare occurrence in crystalline form, dissolves in either acid with effervescence.

The fact that each mineral crystal is often closely incased in a resistant, nearly impermeable membrane may make it necessary to free the face of the mineral from its coating before the test can be applied. This may be done by holding a thin section in a pair of forceps, igniting, and allowing to burn or char only sufficiently to free the crystal, or a portion of it. Care must be exercised, for the calcium oxalate, with sufficient heat, is changed to calcium carbonate and finally to calcium oxide.

H. CHEMICAL GROWTH-RINGS

Some woods without any visible structural growth-rings, may yet exhibit what appear to be definite seasonal variations in the chemical composition of the wood, so that distinct chemical rings

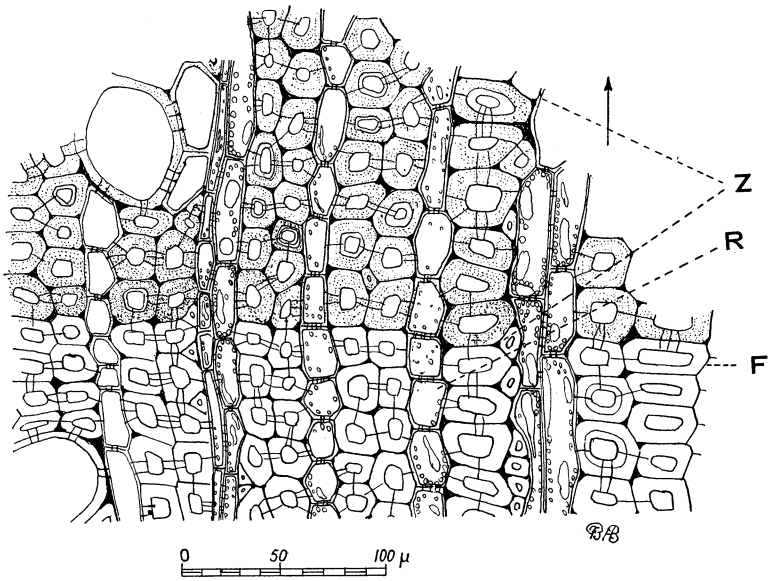

FIG. 6. Chemical growth-ring in *Xylosma hawaiense* Seem. Arrow indicates direction of growth. *Z*, lignified zone. A slight retardation of growth is indicated by somewhat shortened ray-cells at *R*, and occasional slightly compressed fibers (*F*).

may be demonstrated by microchemical means. The term chemical growth-rings is here suggested for such zonal variations in the

cellulose, lignin, mucilage, or other components of the cell-wall, which appear correlated with seasonal growth. The evidence that such zones of chemical variation actually represent true growth-rings is not without structural confirmation. For example, on following carefully a chemical ring (FIG. 6) which appears sharp on its outer face, as in *Xylosma hawaiiense* Seem., such evidences as the presence of short ray cells (*R*) or slightly flattened fibers (*F*) would seem to indicate a slight retardation of growth at that place.

In case of woods in which chemical rings are present due to seasonal variations in lignification, such rings are readily demonstrated by placing cross-sections 15–20 μ thick in an alcoholic solution of phloroglucinol for two minutes, then treating with strong hydrochloric acid. Definite chemical rings appear, which are sharply defined on the outer face, blending gradually into deeper shades toward the interior. Similarly, the cellulose test may reveal zonal differences in the amount of cellulose. Another type of growth-ring is defined by seasonal zones of mucilage-reacting fibers, but such zones, though occurring in regular zonal alternation, may not be sharply defined on either face. In *Alphitonia excelsa* Reiss., such mucilaginous chemical rings are plainly visible without a lens.

7. LIQUID PENETRATION TEST

It not infrequently happens that tyloses, gums, and other bodies which may fill the lumina of vessels and other conducting elements in the region of the heartwood, are displaced or lost during the process of sectioning or staining, so that, from a microscopic examination, one may fail to make an accurate estimate of their presence or abundance. The following test is of material aid: Alcohol is dropped upon the transverse surface of a dry block of wood. If tyloses, gums, or other bodies are abundant, the liquid spreads out over the surface; if absent, the liquid quickly disappears in the tissues and soon runs through to the opposite end. Thus, in case of the red oaks, only a few seconds are required for the liquid to appear at the opposite end of blocks six inches or more in axial thickness. Woods, in general, separate rather definitely into two classes, those which are penetrable and those which are not.

8. SUMMARY

In cutting thin woody sections with a sliding microtome, it is essential, for accurate results, that careful attention be paid to sharpening and adjusting the knife. The blade must be ground to perfect axial alignment. The wedge-shaped edge should have an angle of approximately 20°; for general work this gives better results than a more acute edge.

In softening tissues of tropical woods for sectioning, strong hydrofluoric acid may be used in preference to weak. The length of time required to soften tissues of tropical woods varies from a few days to several weeks, little if any injury being done to the tissues by remaining a long time in acid. The process of demineralization of the cell-wall may be completed without perceptible effect upon the outline of the calcium oxalate crystals contained in the cells, beyond the fact that the refractive properties may be changed.

In the maceration of woody tissues, Schultze's method may be employed with safety if equal volumes of acid and water are used.

Fibers with one or more mucilaginous inner layers are frequent in the xylem of tropical trees. Perhaps the most constant properties of the mucilaginous layers consist (1) in their marked swelling in water and (2) their brittleness on drying. With stains and reagents, reactions differ with the age of the tissue. Thus in young tissue (twigs, sapwood) gathered during the period of rest, a purple color is obtained with zinc chlor-iodide; haematoxylin, anilin blue, or Congo red stain deeply. In old tissue (heartwood), the mucilaginous layers react, as a rule, yellow or brownish with zinc chlor-iodide, and take stains irregularly. Woods in which mucilaginous fibers are abundant may shrink greatly on drying or swell correspondingly on wetting, though not to the same degree as the mucilaginous membranes.

Woods without structural growth-rings may possess chemical rings demonstrable by microchemical means.

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